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Porcine-derived DNA fluorescene PCR detection Kit

Product Number: PDK01

Shipping and Storage

Please store the reagents at -20℃ and avoid repeated freeze-thaw cycles. The validity period is two years; Before use, it should be completely melted at room temperature and thoroughly inverted before centrifugation.

Components

Component	50 tests
Nucleic acid amplification reagents	
Porcine-derived Mixture	1 ml×2
Taq Master mix	30μl×1
Reference substance	
Negative control	50μl×1
Positive control (total DNA of tissues in pork)	50μl×1

Description

This kit utilizes a pair of specific primers for pig mitochondrial DNA, a specific fluorescent probe, and components such as Hot Start Taq DNA Polymerase and four types of monomeric nucleotides (dNTPs). PCR technology is used to amplify conserved genes in pig mitochondrial DNA, and external standard methods are used to detect mitochondrial DNA in the sample. The lower limit of DNA detection is 0.1pg/μl.

This reagent kit has no non-specific amplification for samples from cows, horses, sheep, chickens, ducks, rabbits, donkeys, mice, and geese.

Reagents and items that users need to bring themselves

1. 1.5 ml centrifuge tube, 8-row or single tube PCR tube
2. Pipette and suction head (To avoid contamination between samples, please choose a pipette suction head containing a filter element)
3. Disposable gloves, protective equipment, and tissues
4. Desktop small centrifuge (can be equipped with rotors for centrifuging 1.5 ml centrifuge tubes and 2 ml centrifuge tubes)
5. Vortex oscillator

Note

1. Before use, completely dissolve the reagent at room temperature, mix it upside down and centrifuge briefly to allow the reagent to deposit to the bottom of the tube.
2. When preparing PCR reaction solution, please place the reagent on ice and avoid strong light exposure.
3. It is recommended to set at least three experimental areas from the preparation of reaction solution to the addition of detection samples, and conduct physical isolation.
   - 3.1. Areas 1: Preparation and packaging of reaction solution.
   - 3.2. Areas 2: Preparation of DNA for testing samples.
   - 3.3. Areas 3: Add DNA from the test sample to the reaction solution for reaction and detection (PCR reaction tubes after amplification are strictly prohibited from being opened!)

Protocol

1. Each test reaction system is configured as follows:

Reagent	Volume(μl)
Porcine-derived Mixture	34.6
Taq Master mix	0.4

2. Calculate the usage amount of each reagent component based on the sample size to be tested, add it to a 1.5 ml centrifuge tube, mix well, centrifuge briefly, and add 35μl of Porcine-derived Mixture to each of the set n (n=number of samples+1 tube positive control+1 tube negative control) PCR reaction tubes, Transfer to the sample processing area (note that due to errors in the pipette, usually the mixed n samples for Porcine-derived Mixture can only be divided into n-1 PCR tubes. It is recommended to add one tube when calculating the number of detection samples).

   Example: If 5 samples need to be tested, the reagent should be prepared according to the amount of 8 (n+1) Porcine-derived Mixture, that is: Porcine-derived Mixture 34.6×8=276.8μl, then add Taq Master mix 0.4×8=3.2μl, mix well and divide the PCR detection solution into 7 PCR tubes at a rate of 35μl per tube. Discard any excess PCR detection solution.

3. Sampling

   Add 5μl of prepared DNA solution and control substance (usually negative control tube 1 and positive control tube 1) to the PCR reaction tubes, cover the tubes tightly, centrifuge briefly, and place the reaction tubes into the fluorescence PCR detector. Record the order of sample placement.

4. PCR reaction

Cycles	Temperature	Time
1	95°C	1min
40	95°C	15s
	60°C	35s

The fluorescence signal collection (Detector) is set to FAM, the reference (Passive) is set to None, and the data collection is set at 60 °C

Note:This product does not contain Rox reference. If the instrument used requires Rox as a reference or if you want to achieve better curve results, you can order 50×ROX Reference Dye/50×ROX Reference Dye II separately

Result analysis:

1. Threshold setting

   The threshold is set to 500 (imported instruments such as ABI, Roche, Bio Rad, Agilent Technologies do not need to be set).

2. Quality control standards

   2.1. The detection limit of this reagent kit for Porcine derived component DNA is 0.1 pg/μl.

   2.2. Negative control: Ct value>38 or no Ct value, linear or slightly diagonal, with no exponential growth period.

   2.3. Positive control: There is a typical amplification curve and the Ct value is ≤ 35.

3. Result judgment

   3.1. If the Ct value of the sample test result is ≤ 35 or there is a significant exponential growth period, it can be directly judged as the detection of pig derived DNA components.

   3.2. The Ct value of the sample test result is within the range of 35-38. At this time, the sample should be tested repeatedly. If the Ct value of the repeated experiment result is still within the range of 35-38 and there is a significant exponential growth period, it is judged as positive, otherwise it is considered negative.

   3.3. If the Ct value of the sample test is greater than 38 or there is no Ct value, the linear shape is a straight line or a slight diagonal, and there is no exponential growth period, it can be directly judged as "no detected Porcine derived DNA component".